Mouse models in quest for treatment(s) of MCOPS12

The 3rd MCOPS12 Patient / Family Meeting
11-12 September, Salzburg, Austria

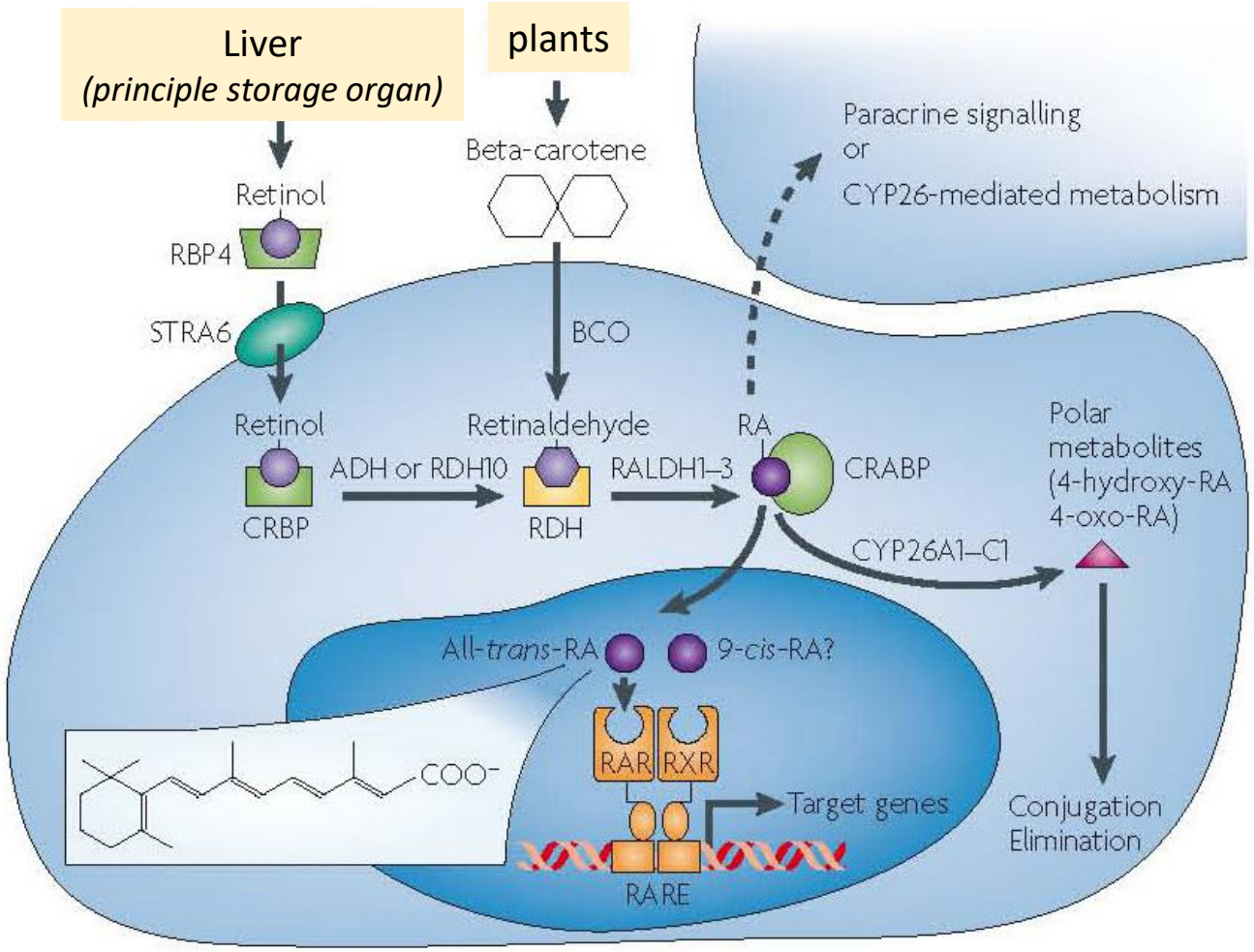


Wojciech KRĘŻEL

Institute of Genetics and Molecular and Cellular Biology

STRASBOURG / ILLKIRCH, FRANCE

Complexity of vitamin A signaling – a secret of its diverse functions



Enzymes convert inactive precursors (retinol, carotenoids) **into active retinoids**

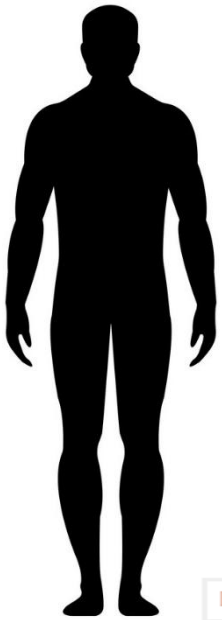
Nuclear Receptors:

- **Retinoic Acid Receptors** α, β, γ
- **Retinoid X Receptors** α, β, γ
- other dimerisation partners of RXRs**: PPARs, TRs, VDR …**

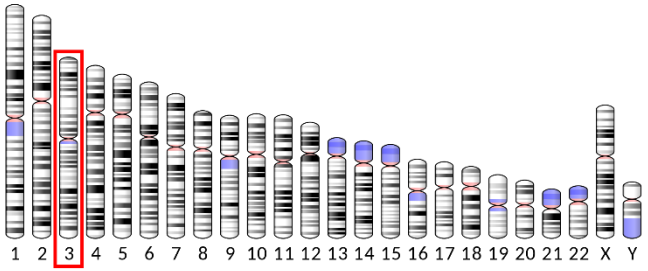
Toolbox (loss/gain of function) **: pharmacology / genetic mouse models / viral vectors / biological chemistry**

RARβ - high conservation between human and mouse (sequence)

Retinoic acid receptor beta (RARβ)



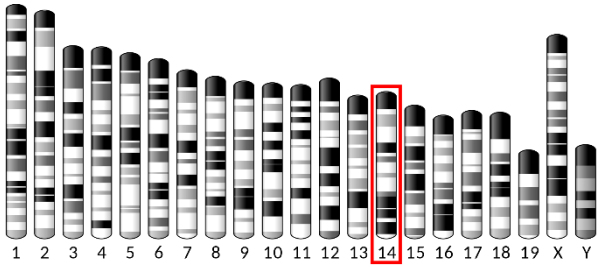
RARβ



RARβ 455 amino acids



Rarβ



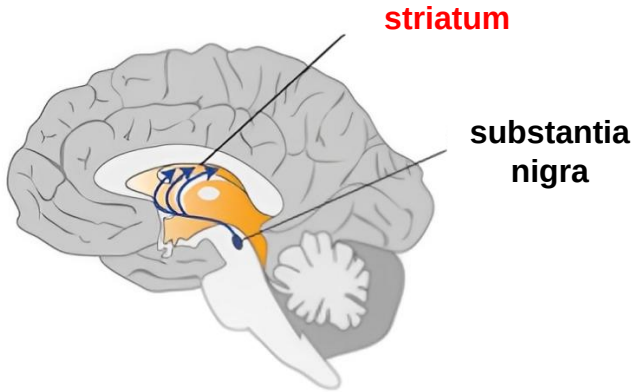
RARβ 482 amino acids

P22605	RARB_MOUSE - **Retinoic acid receptor beta** Mus musculus (Mouse)
E-value: 0.0 Score: 2333 Ident.: 92.3% Positives : 93.2% Query Length: 455 Match Length: 482	98% Similarity



RARβ is expressed in the Striatum

Human Brain

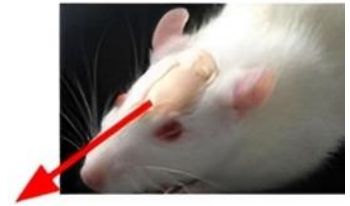


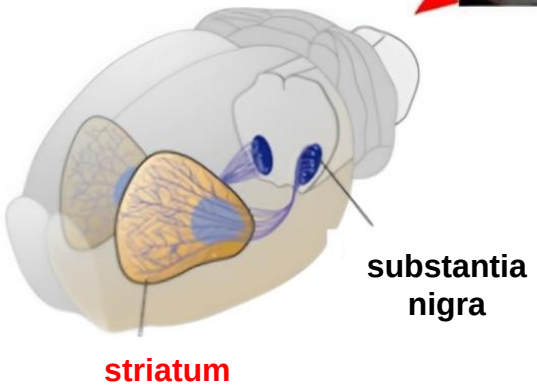
Motor control

Reward processing

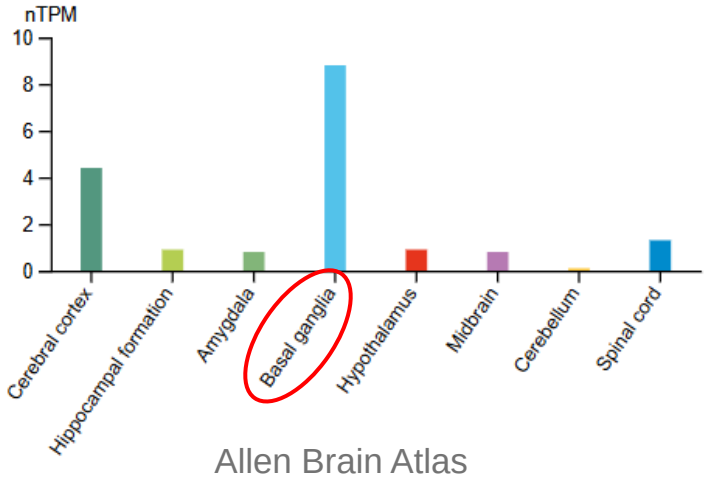
Cognitive tasks

Mouse Brain



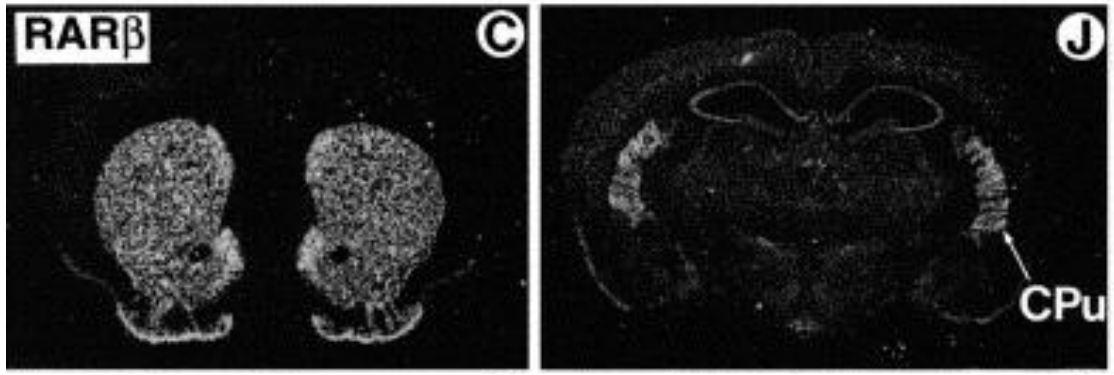


Human brain RARB expression (GTEx RNA-Seq)



Allen Brain Atlas

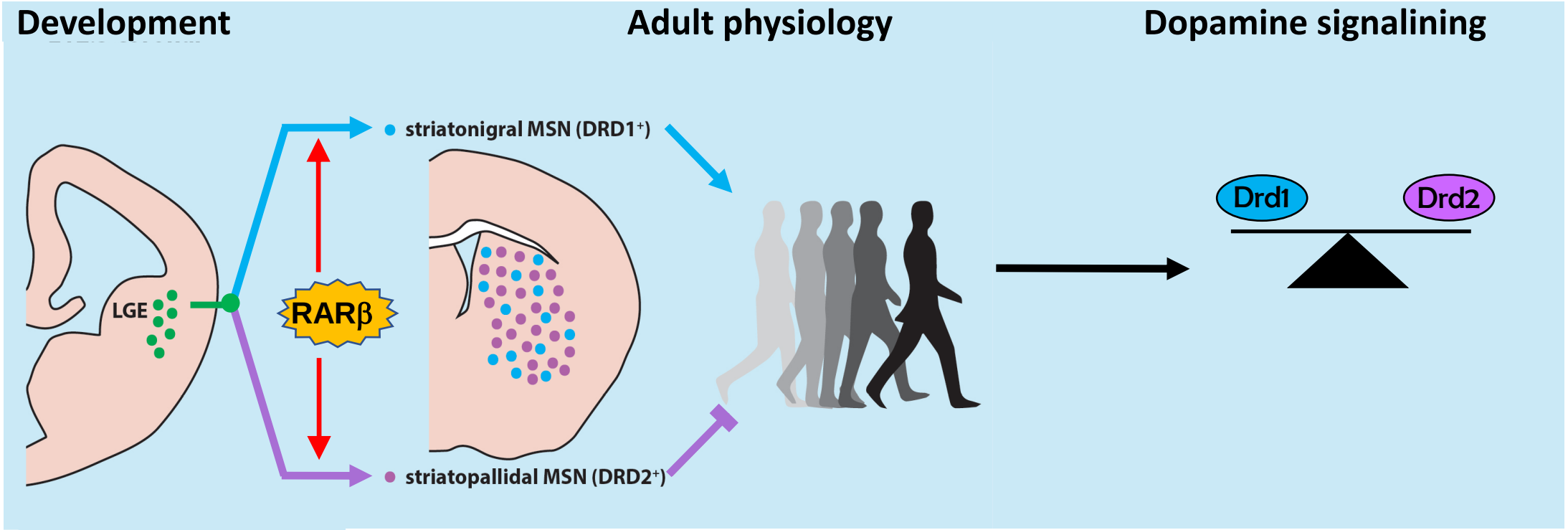
Mouse brain RARB expression (In Situ Hybdridization)



Krezel et al., 1999

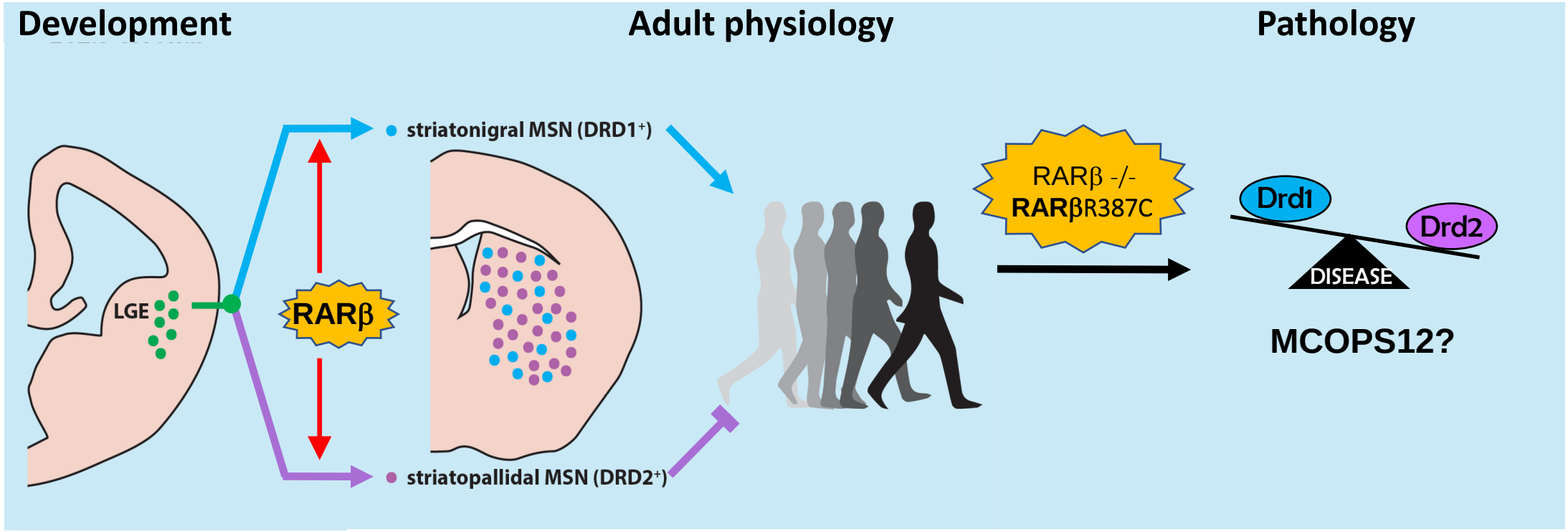
	RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
Striatum						
Caudate–putamen	–*	+++	++	–*	+	+++
Accumbens nucleus core	–*	+++	++	–*	+	++
Accumbens nucleus shell	–*	+++	+	–*	+	+++

Retinoids, RARs/RXRs, and striatal functions



- Striatal functions : motor control, cognitive functions, habits, affect…
- Striatum controls brain activities through balanced signaling of striatonigral and striatopallidal project **M**edium **S**piny **N**eurons (MSNs)
- Dopamine controls both types of MSNs through Drd1 and Drd2 receptor

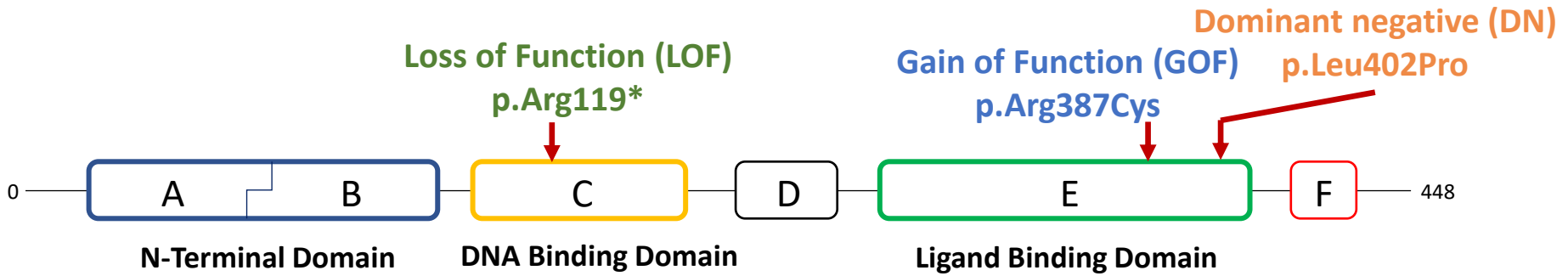
Retinoids, RARs/RXRs, and striatal functions



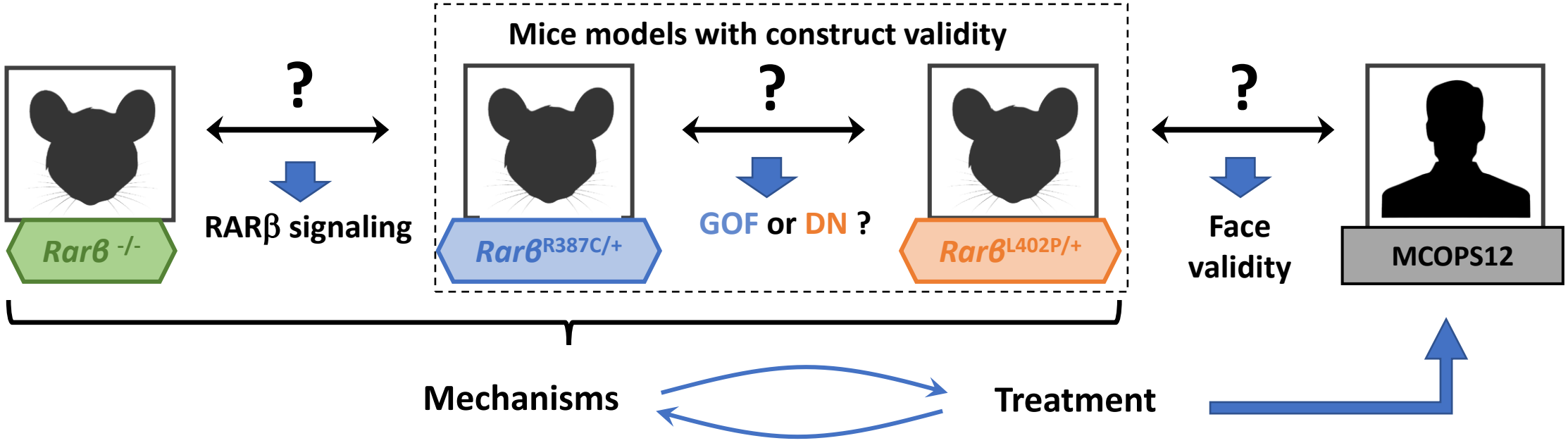
- Do RARβ mutations lead to unbalanced dopamine signaling and striatal functions ?

- A NEED FOR AN EXPERIMENTAL MODEL

Allelic series of RARβ mutant mice models to study MCOPS12



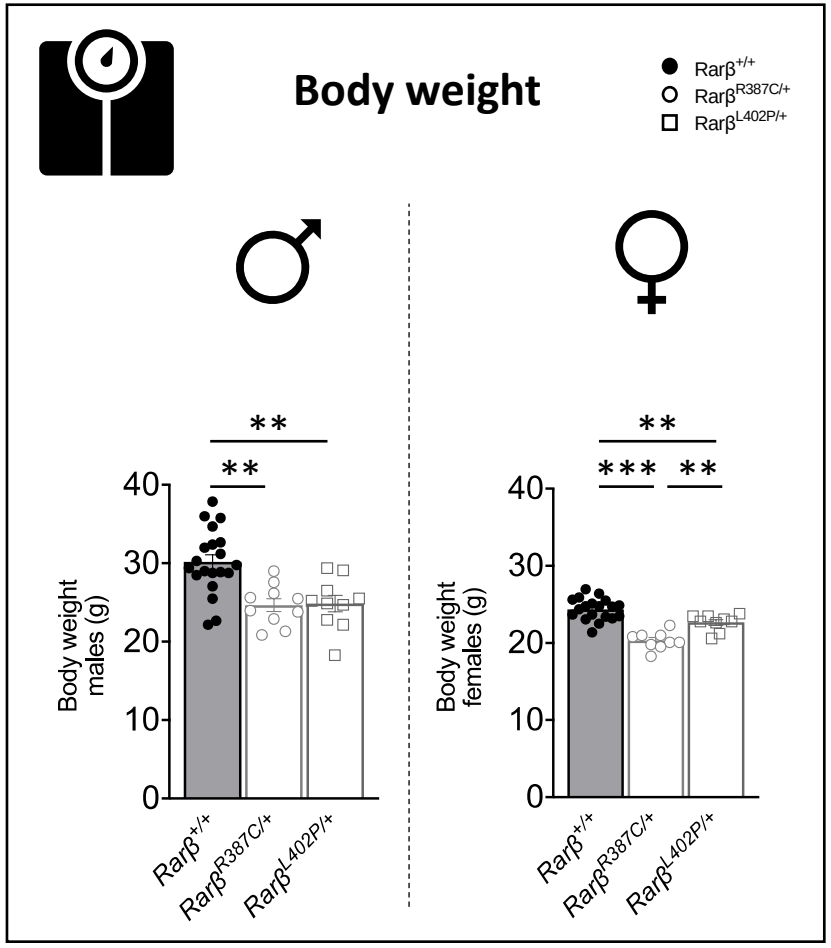
Caron et al., 2023

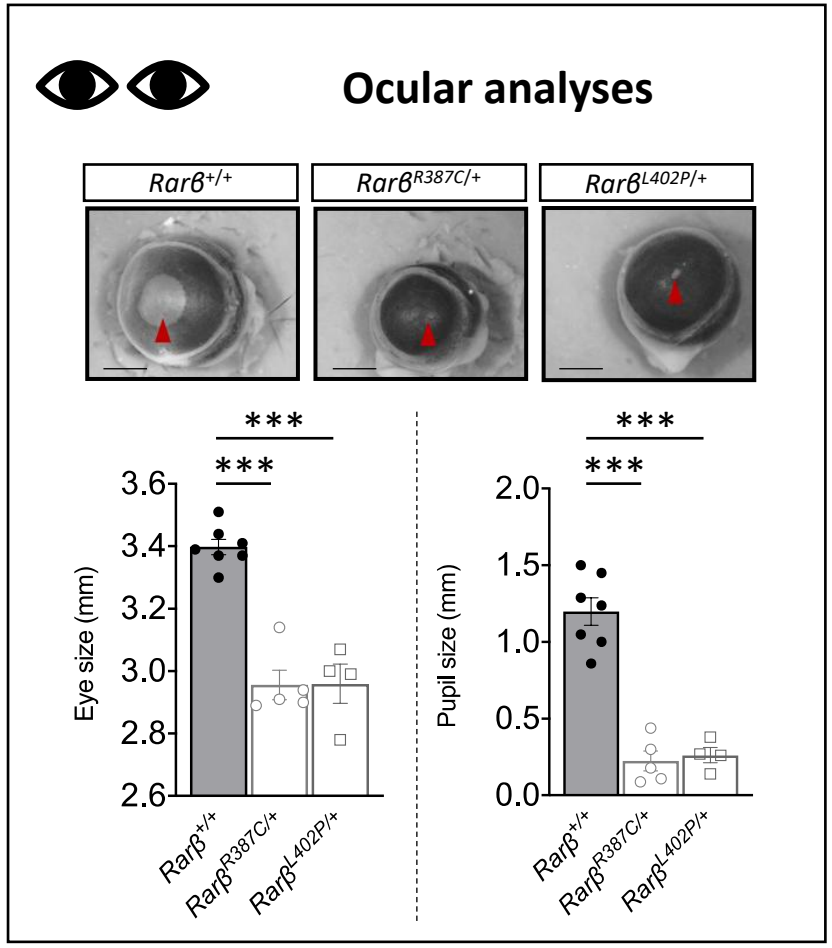


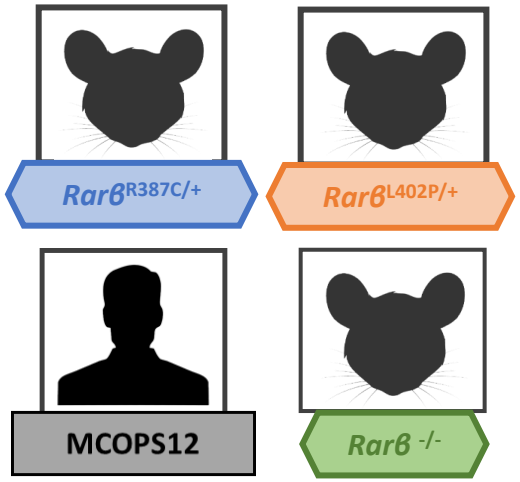
Allelic series of RARβ mutant mice reproduce key MCOPS12 symptoms



Nicolas Zinter



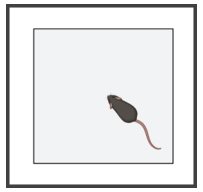


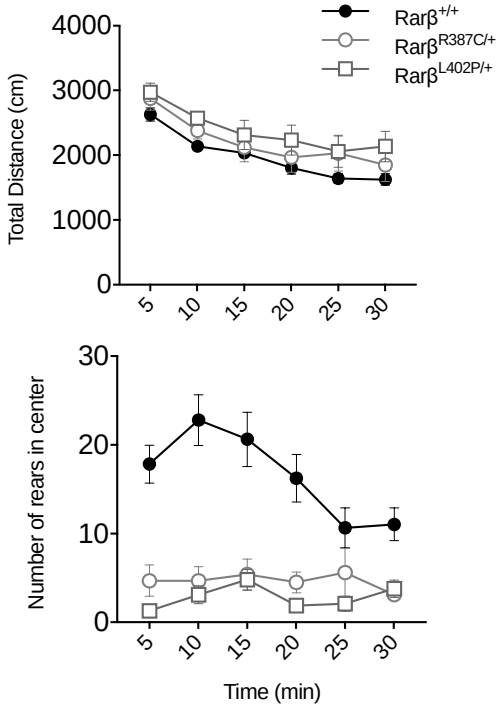


- **Males & females display lower body weight (~20%)**
- **Microphtalmia (<u>absent in *Rarβ* $^{-/-}$ mice</u>)**

Allelic series of RARβ mutant mice reproduce key MCOPS12 symptoms - behavior

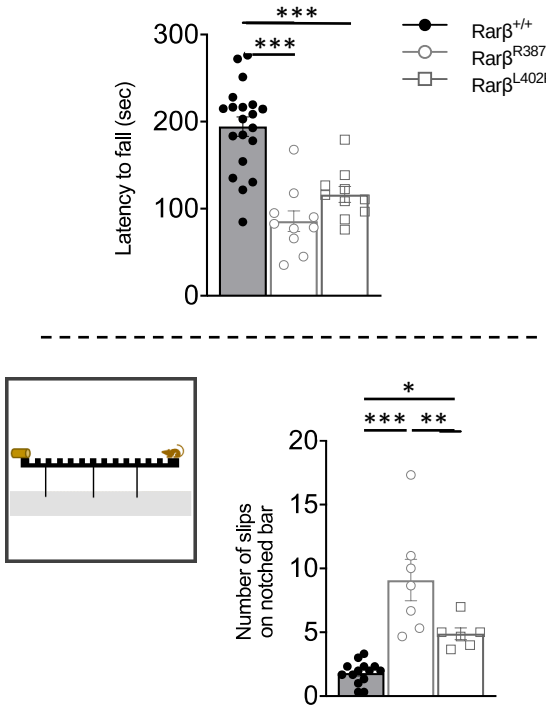
Novelty-induced locomotor activity





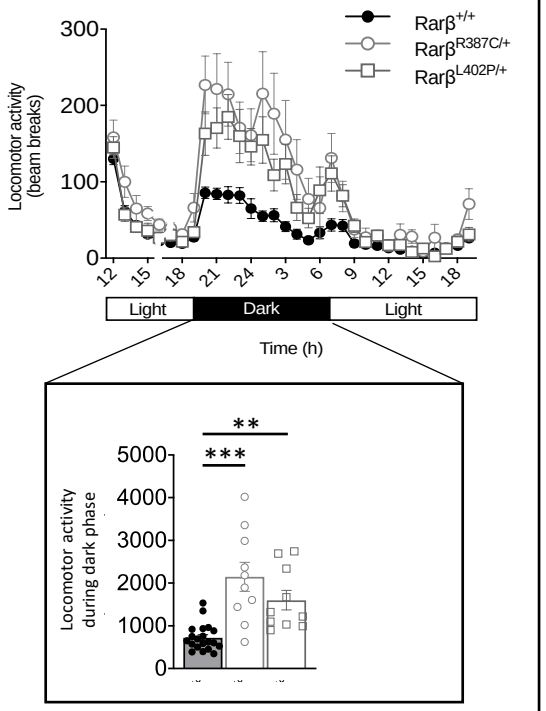
Motor coordination

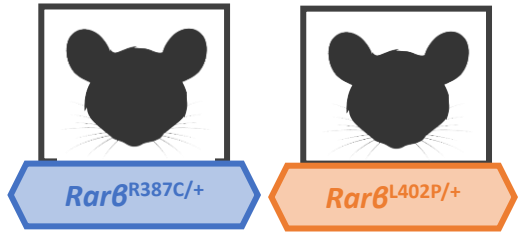




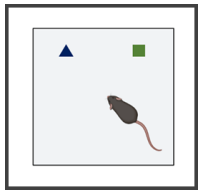
Spontaneous locomotor activity







Cognition/Memory



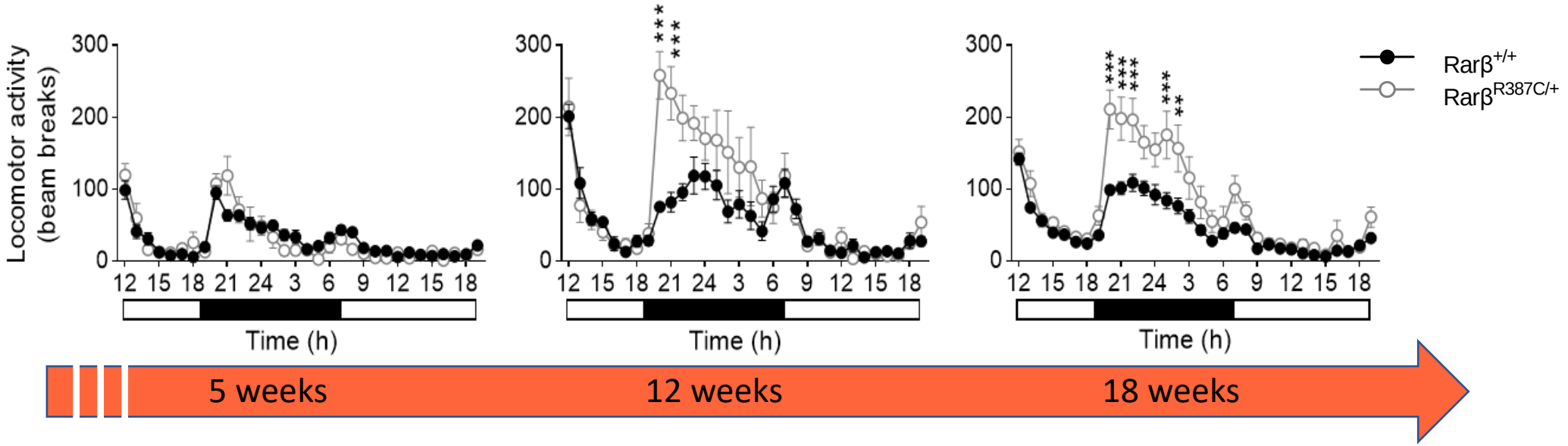
Motor coordination deficits

Spontaneous hyperactivity during night phase of light/dark cycle

Memory deficits

Progressive behavioral deficits point to a window of opportunity for MCOPS12 treatment

An exemple of progressive behavioral abnormalities in *Rarβ*$^{R387C/+}$ mice - hyperactivity



Progressive phenotypes in mice models *(age of onset)*:

- Sponatneous hyperactvity *(8-10 weeks)*
- Memory deficits *(8-10 weeks)*
- Growth defcits *(3-4 weeks)*

Developmental phenotypes in mice models *(age of onset)*:

- Microphtalmia *(pre-natal)*
- Coordination deficits *(post-natal day P0 - P21?)*

❖ ***RARβ***$^{R387C/+}$ **mice display more severe phenotype than RARβ LOF**

❖ **Some phenotypes are progressive**

Zinter et al. BRAIN in press

Drug repurposing program - *hypothesis driven*

Valid animal models of disease



Mechanisms ?

(… and related biomarkers)

Behavioral changes *(disease symptoms = functional readouts)*



↓ Motor Coordination

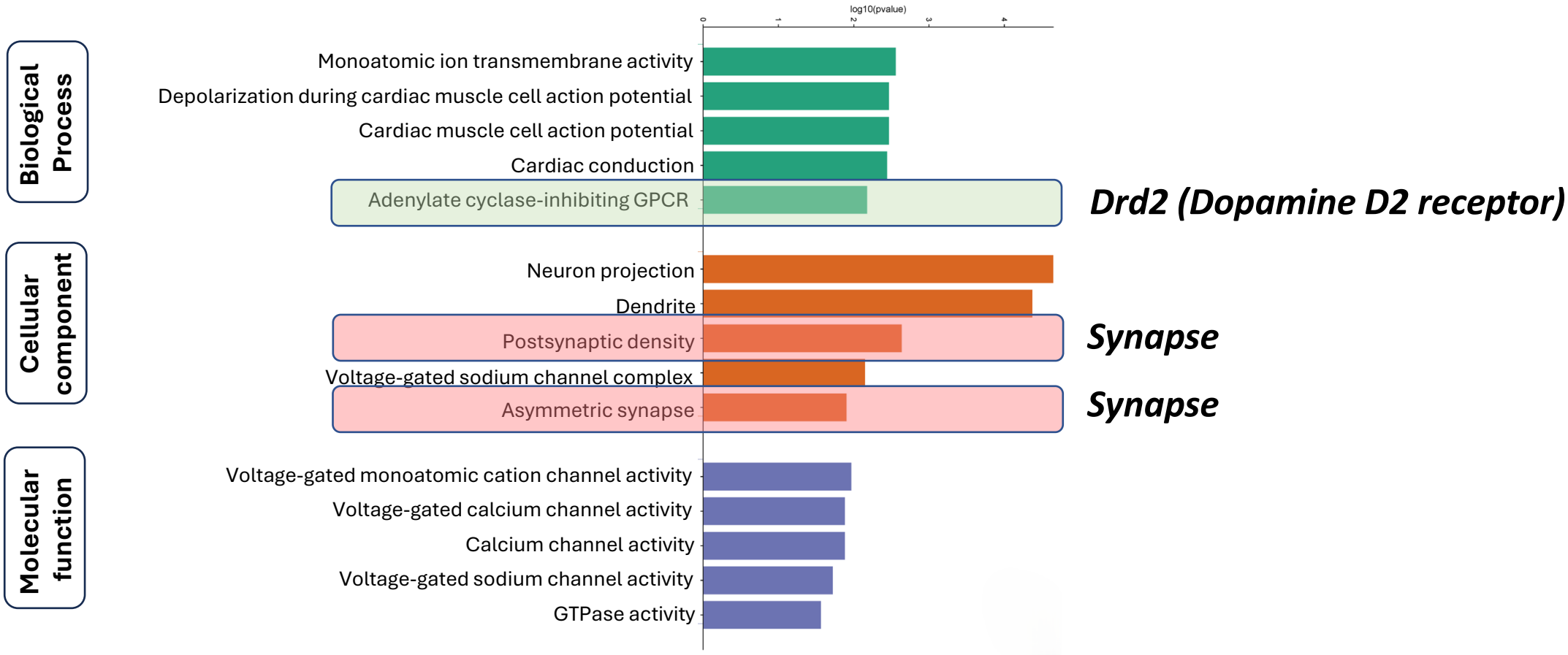
↑ Locomotor Activity

↓ Memory

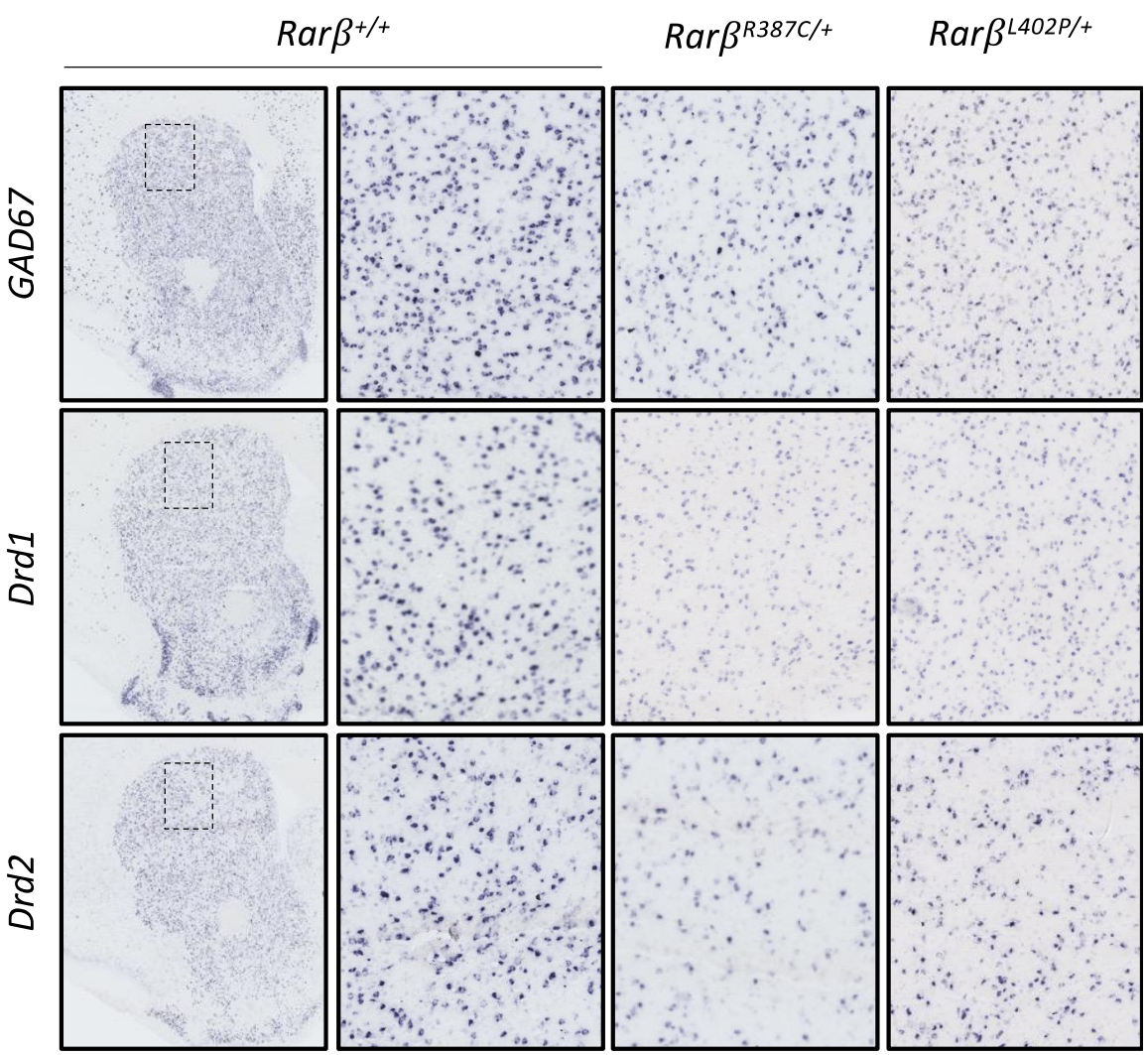
Mechanisms - Genes differentially expressed across mouse models *(mRNAseq, striatum)*

		Number of DEG*	*Rarb*$^{R387C/+}$	*Rarb*$^{L402P/+}$	*Rarb*$^{-/-}$	*Rarb*$^{+/-}$
Upregulated genes	*Rarb*$^{R387C/+}$	*979*	NA	461 (47%)	122 (12%)	7 (0.7%)
	Rarb$^{L402P/+}$	*1186*	461 (39%)	NA	198 (17%)	12 (1.0%)
	Rarb$^{-/-}$	*411*	122 (30%)	198 (48%)	NA	39 (10%)
	Rarb$^{+/-}$	58	7 (12%)	12 (21%)	39 (67%)	NA
Downregulated genes	*Rarb*$^{R387C/+}$	*1386*	NA	777 (56%)	213 (15%)	15 (1.1%)
	Rarb$^{L402P/+}$	*1334*	777 (58%)	NA	253 (19%)	21 (1.6%)
	Rarb$^{-/-}$	*390*	213 (55%)	253 (65%)	NA	31 (8%)
	Rarb$^{+/-}$	*41*	15 (37%)	21 (51%)	31 (76%)	NA

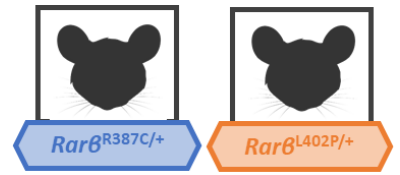
Mechanisms - Gene Ontology (GO) analysis of downregulated direct targets of Rarb

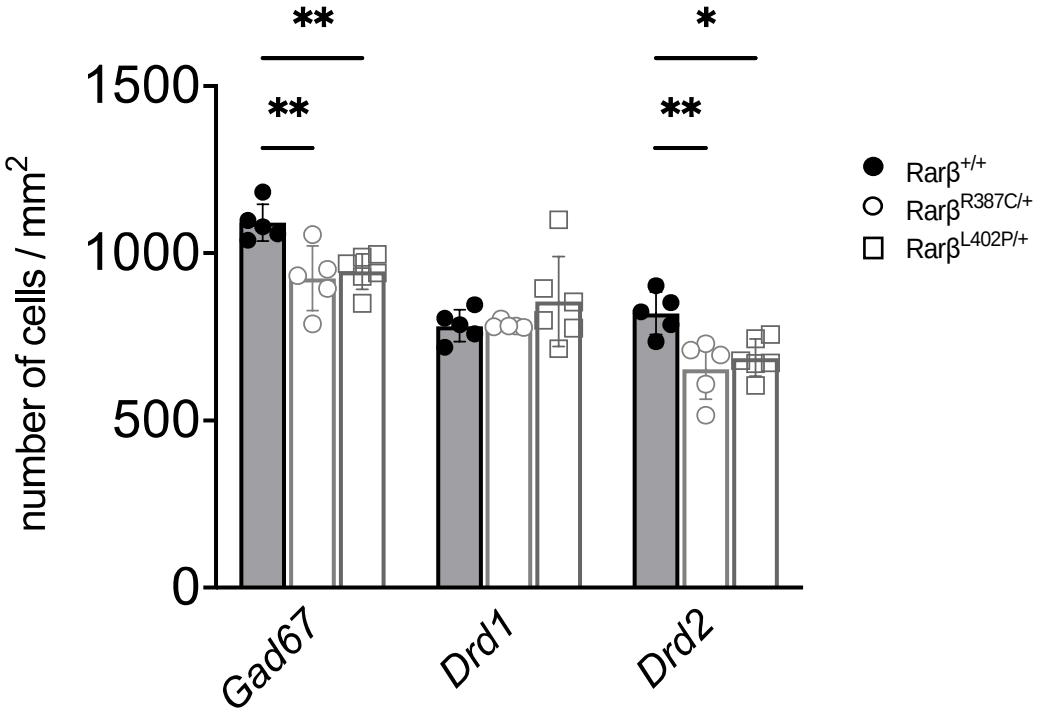


Mechanisms - Reduced number of Drd2+ spMSN in MCOPS12 allelic series

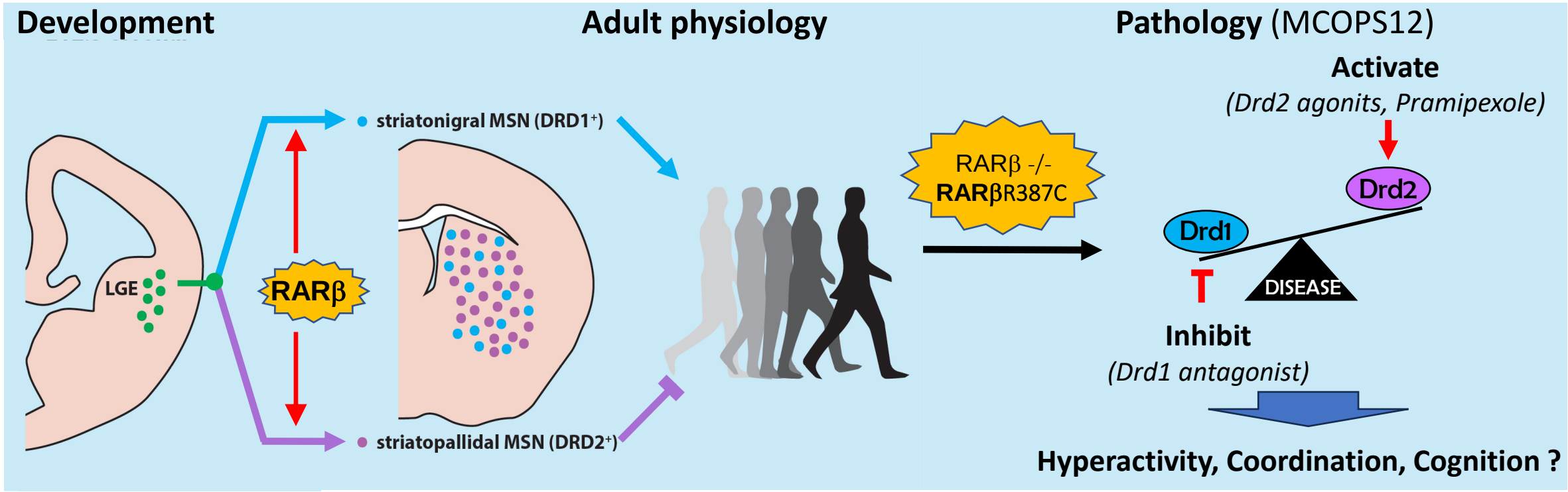


Striatum *(in situ hybridisation)*



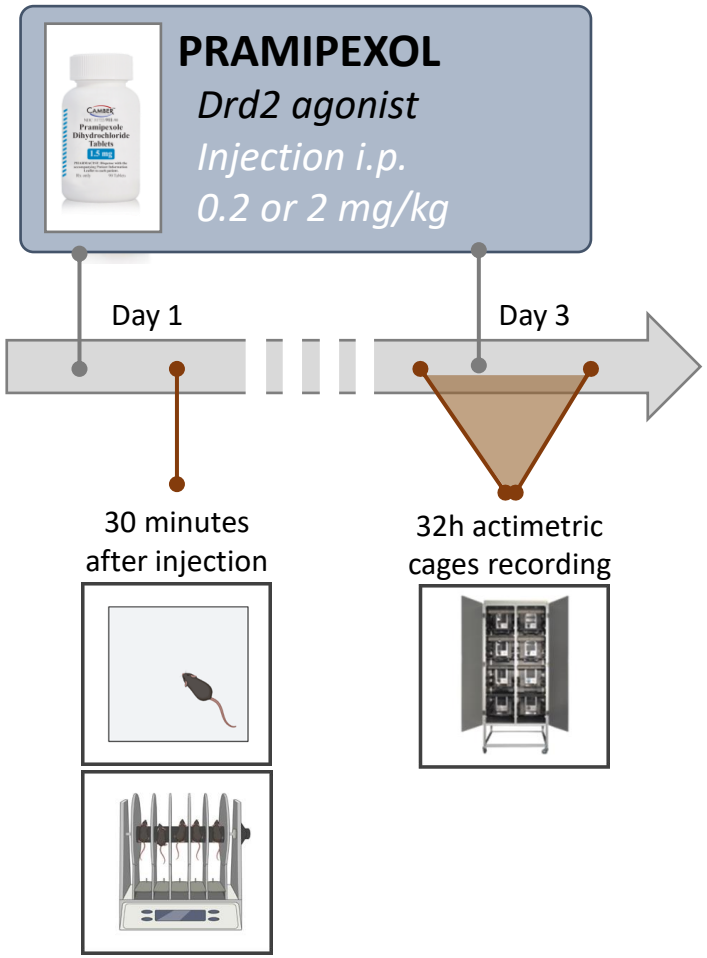


Compromised Drd2+ spMSN signaling leads to unbalanced dopamine modulation of MSNs



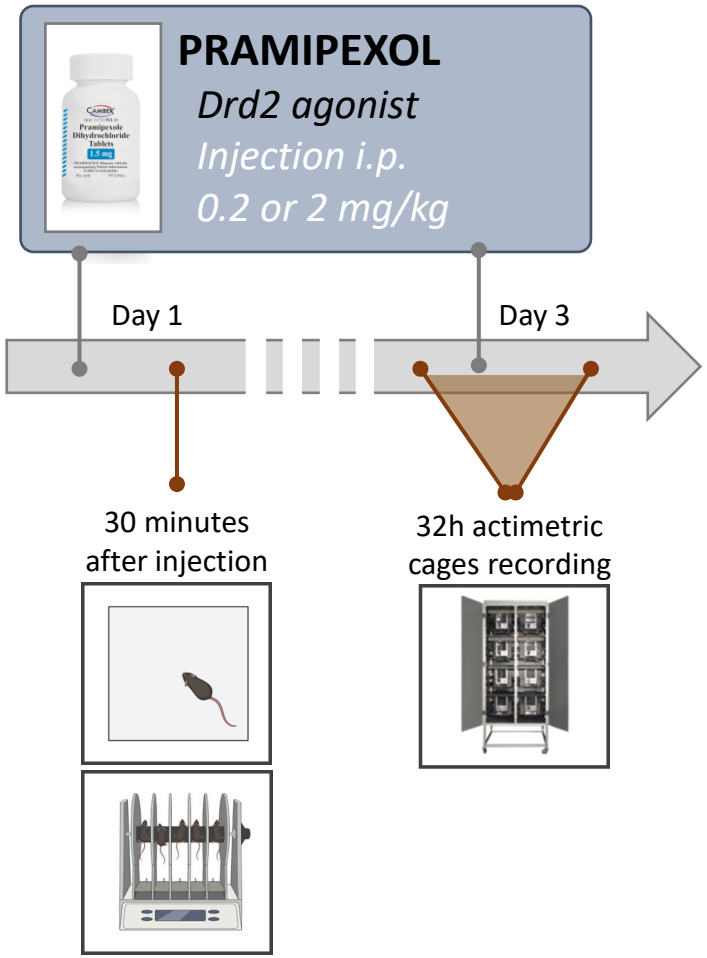
- Reduced number of Drd2+ MSNs
- Reduced signaling of Drd2+ MSNs (blunted response to Drd2 antagonist, amphetamine) but not Drd1+ MSNs
- *Rarb*$^{Drd2-/-}$ recapitulate motor coordination deficits (Drd2-dependent ?) but not hyperactivity (Drd1-dependent ?)

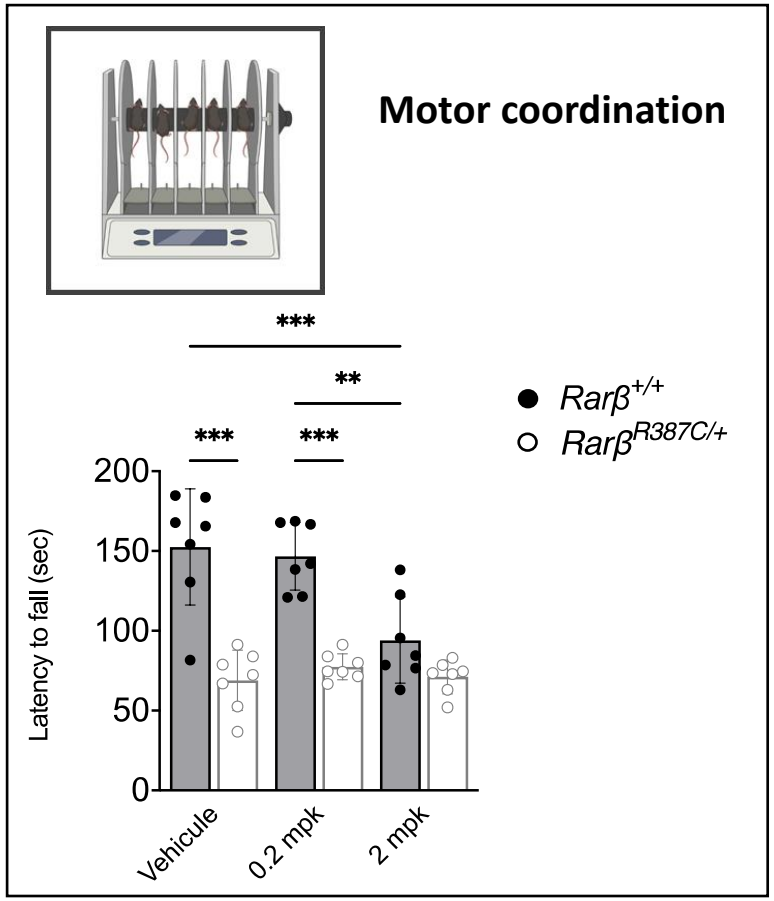
Treatment - Enhancing Drd2 signaling in *Rarb*$^{R387C/+}$ mice

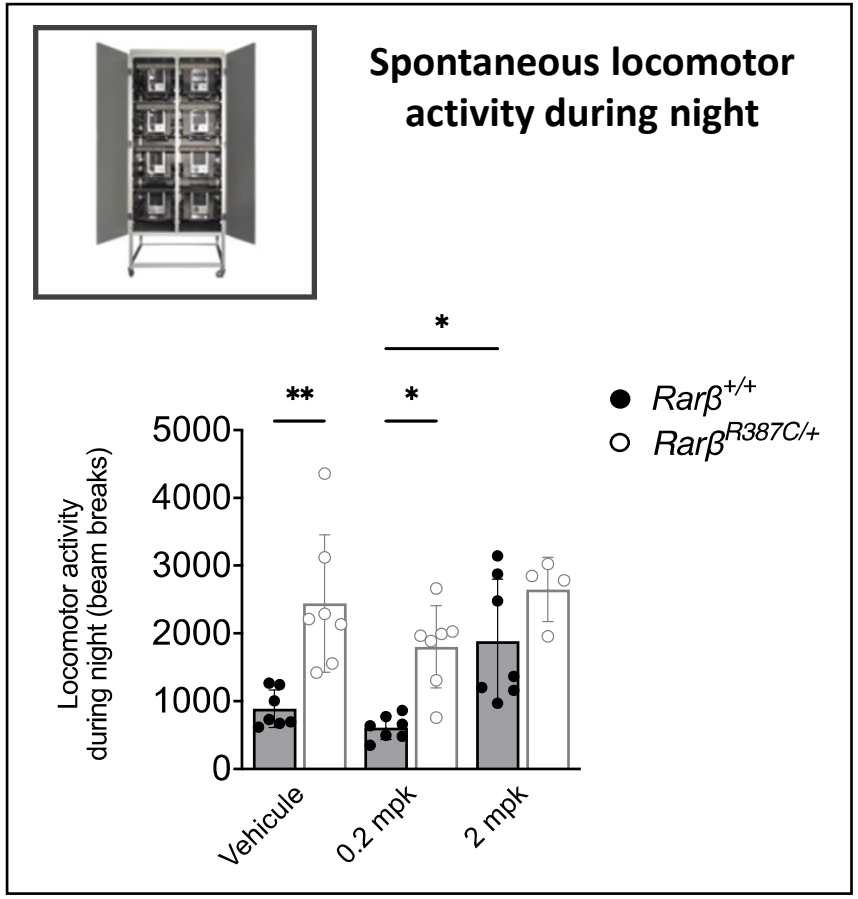




Treatment - Enhancing Drd2 signaling in *Rarb*$^{R387C/+}$ mice







Motor coordination deficits are not rescued by pramipexol treatment.

Hyperactivity is not rescued by pramipexol treatment.

❖ ***Drd1 pharmacology screen in progress***

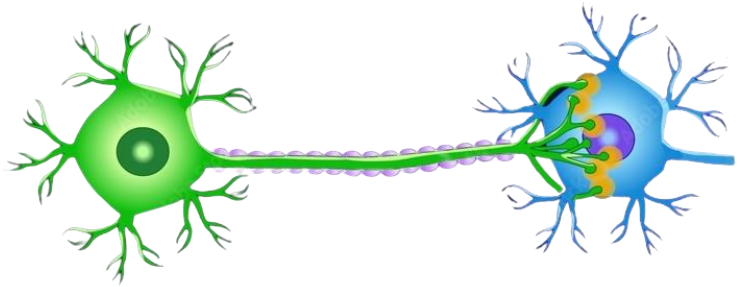
Mechanism - Investigating Medium Spiny Neuron SYNAPSE





Hypothesis :

MCOPS12-causing RARβ variants alter striatal cellular communication at SYNAPSE level

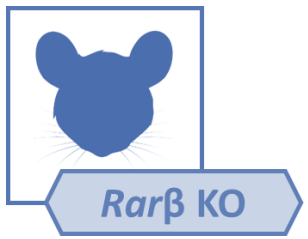


Objective :

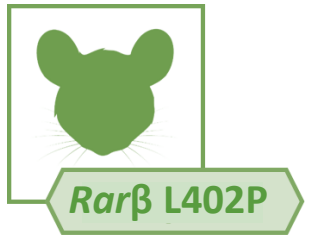
Proteomic analyses of synaptic compartments

Mechanism - Investigating Medium Spiny Neuron SYNAPSE









Striatum Collection

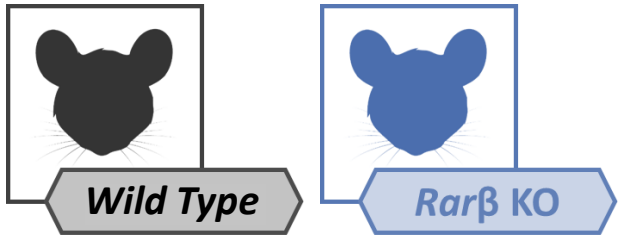


Synaptosomes isolation



Proteomics
Mass spectrometry

Mechanism - SYNAPSE preparation



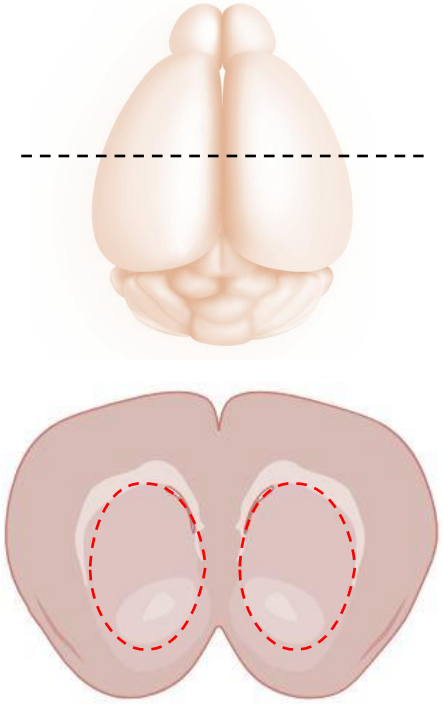
Step by Step Workflow



Wild type



RarβKO



Striatum dissection

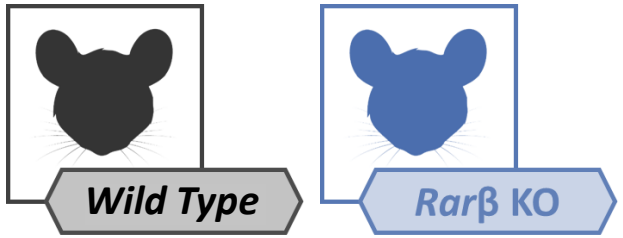


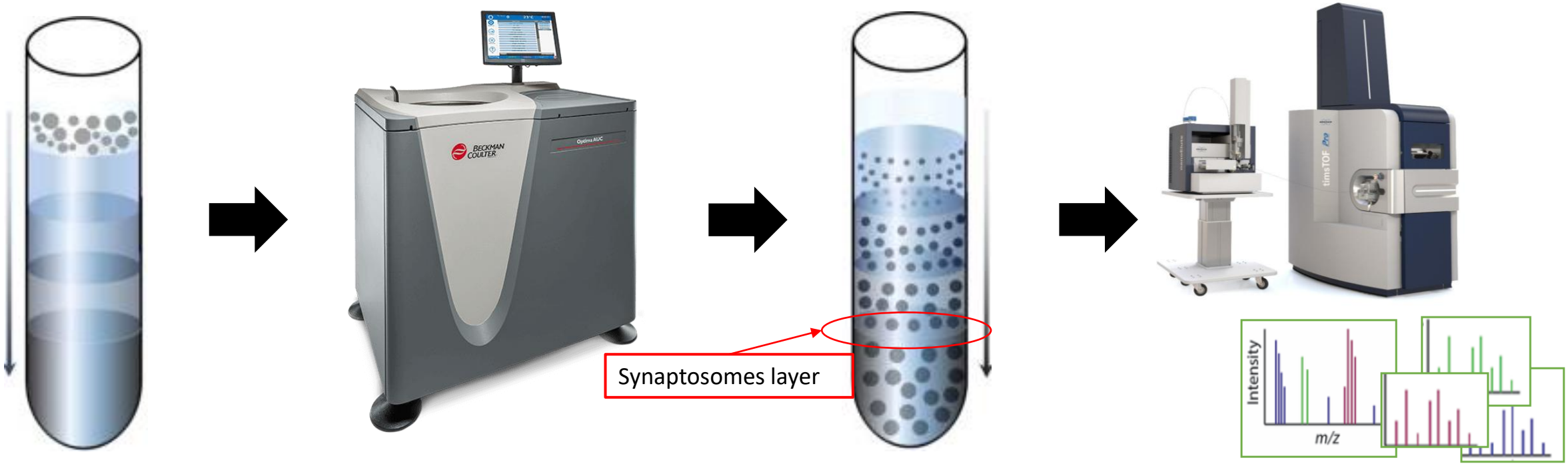
Homogenization



Sucrose gradient

Mechanism - SYNAPSE preparation





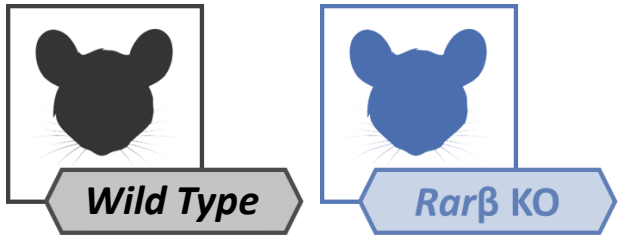
Sucrose gradient

Ultracentrifugation

Separation

Mass-spectrometry

Mechanism - SYNAPSE preparation



Validation of the technique:

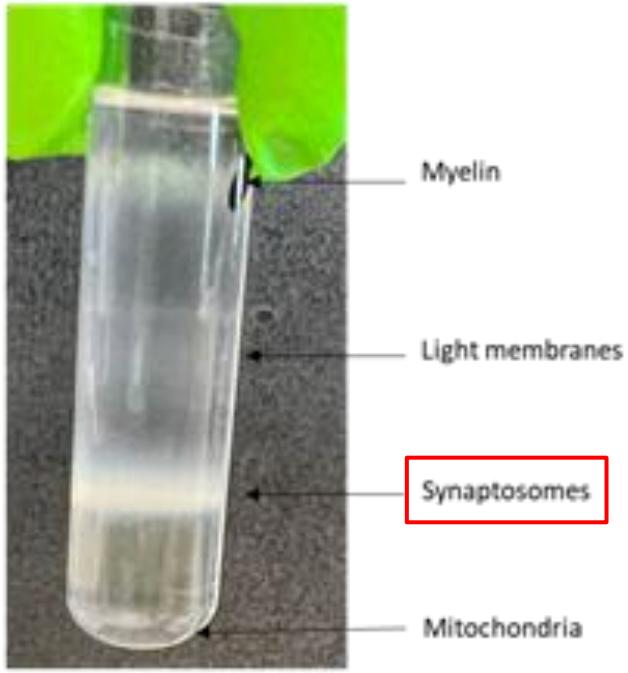
Whole synapses isolation

Sucrose gradient-based separation profile of synaptosome fraction

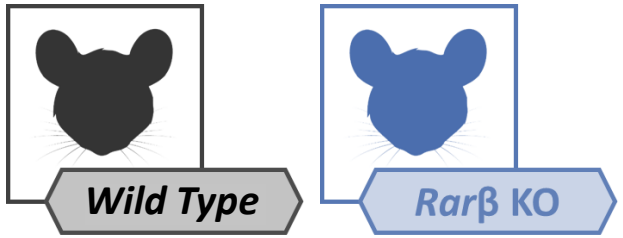
Expected



Obtained

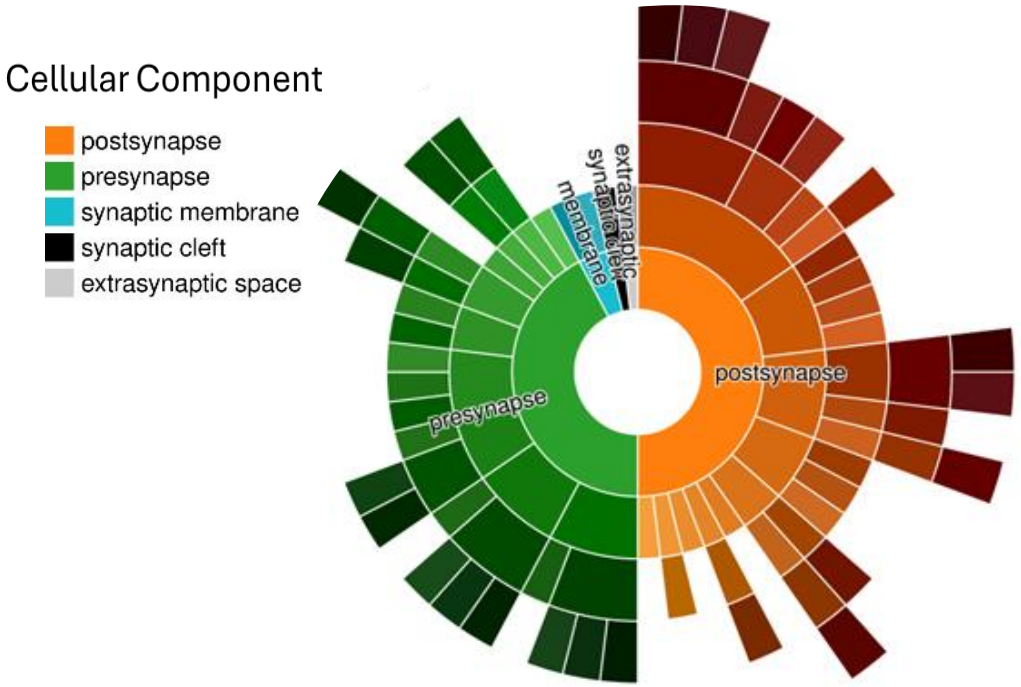


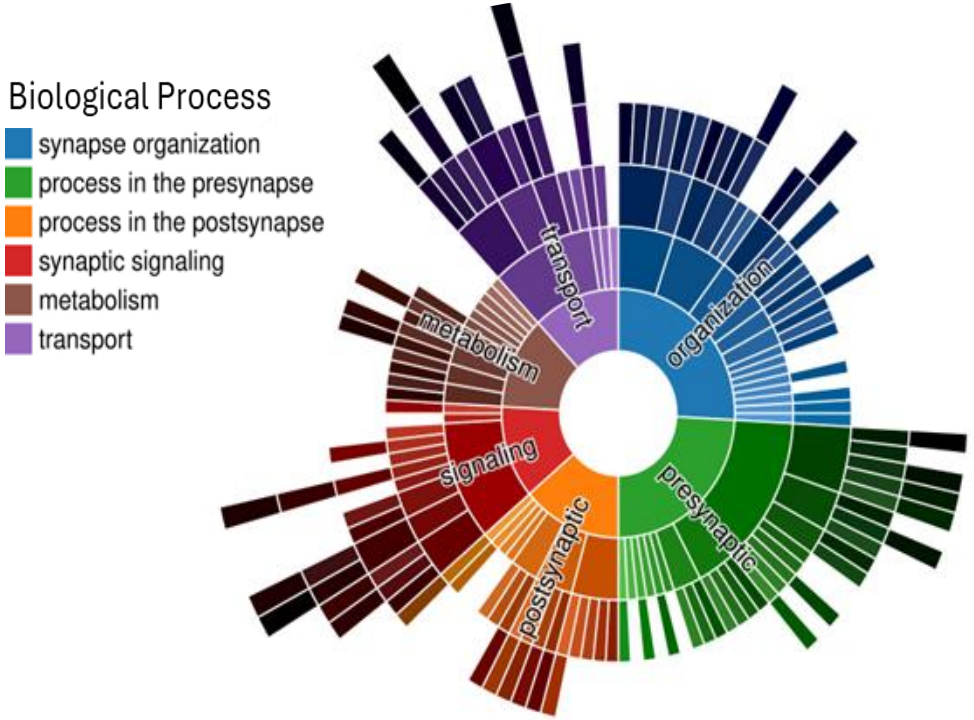
Mechanism - SYNAPSE preparation



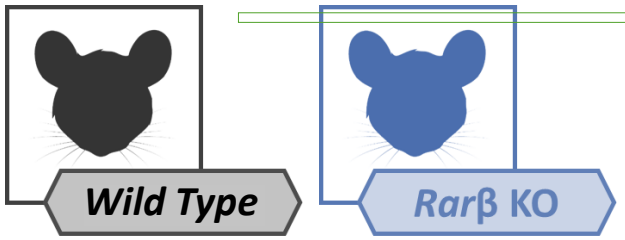
Validation of the purity of the synapse preparation :

Conforming Synaptic protein enrichment analyses using SynGO software





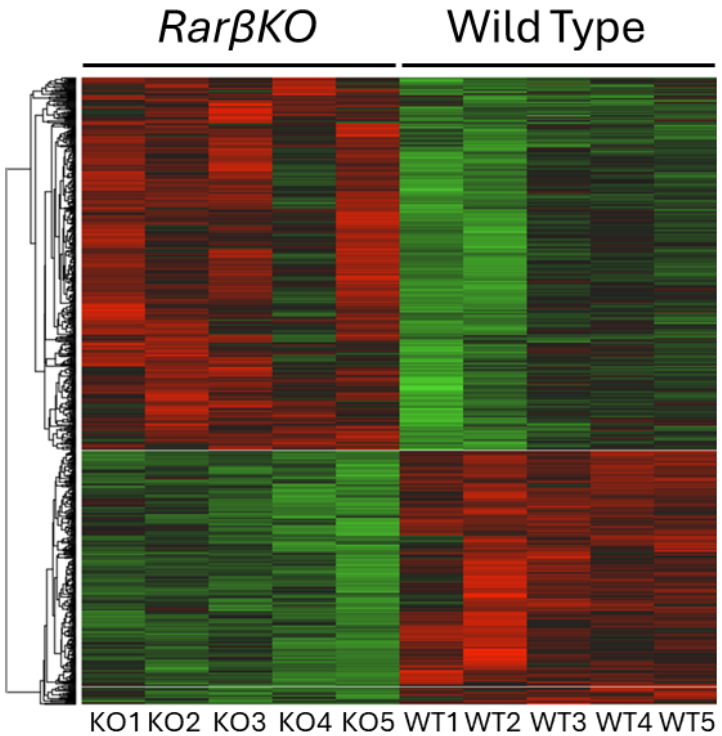
Protein composition of synapse (proteomics)



Differential Synaptosomal Proteome:

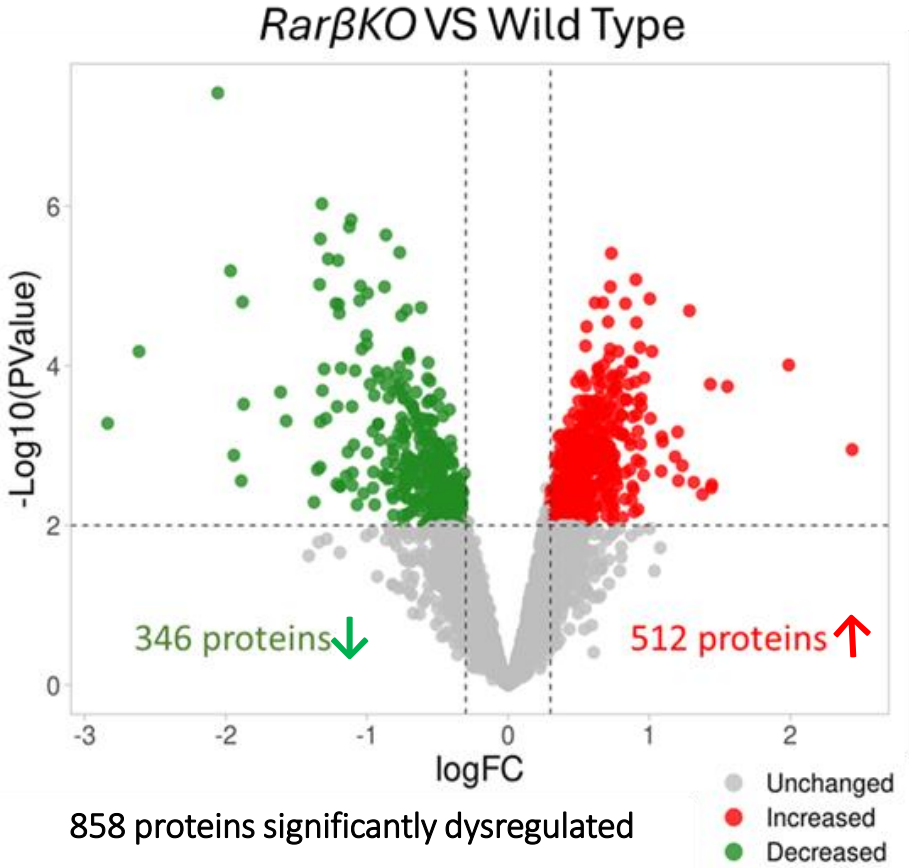
RarβKO VS Wild Type

Hierarchical clustering of differentially expressed proteins



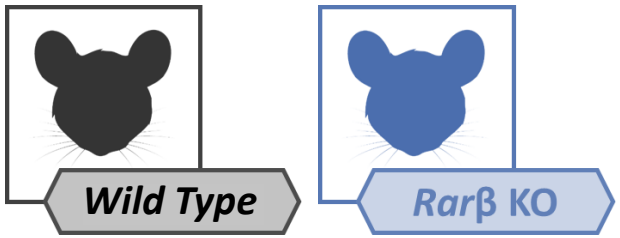
Marked difference between two genotypes and high reproducibility

Differentially expressed proteins in synaptosome fraction of Wild type and RarβKO



858 proteins significantly dysregulated

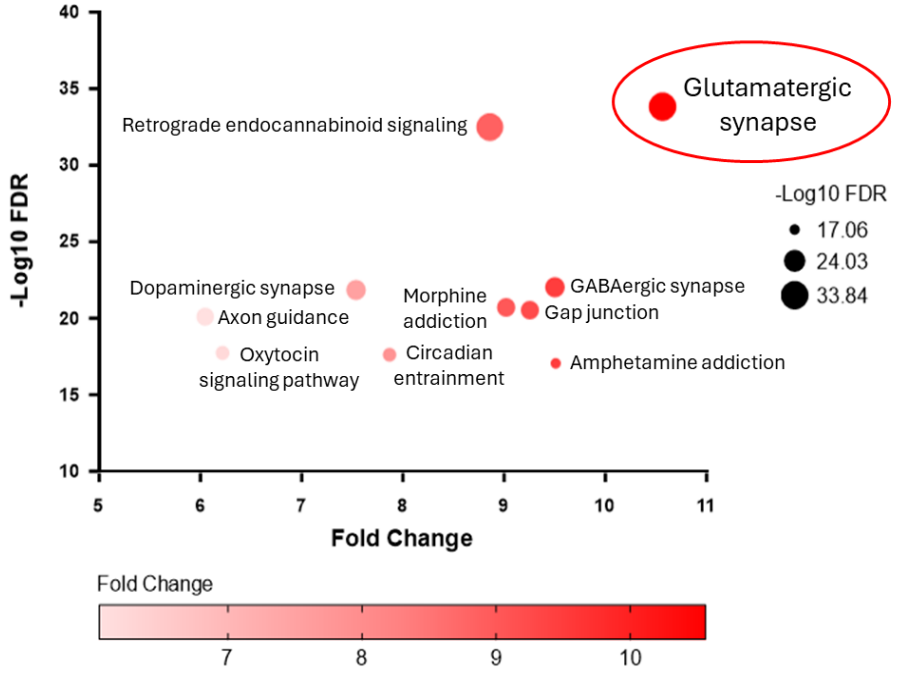
Mechanism - Increased glutamatergic signaling ?



Differential Synaptosomal Proteome:

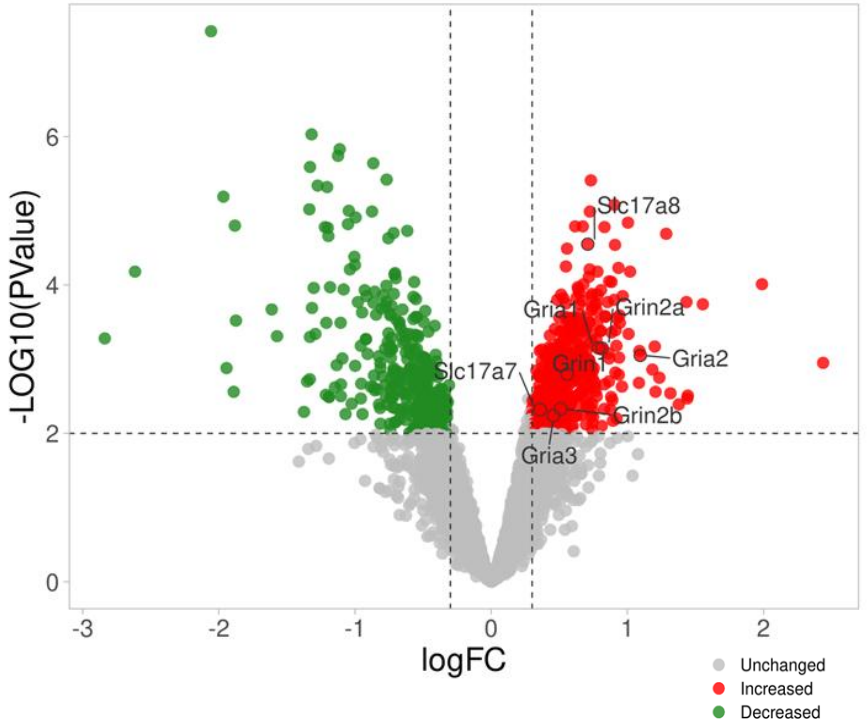
RarβKO VS Wild Type

KEGG pathways enrichment analysis



Glutamatergic pre- and post-synaptic components highlighted

RarβKO VS Wild Type



Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior ?

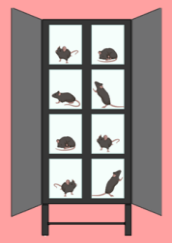
Functional relevance of Glutamatergic signaling upregulation



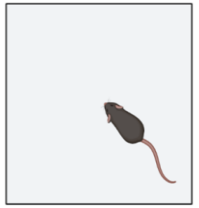
Similar neurological symptoms



Motor Coordination

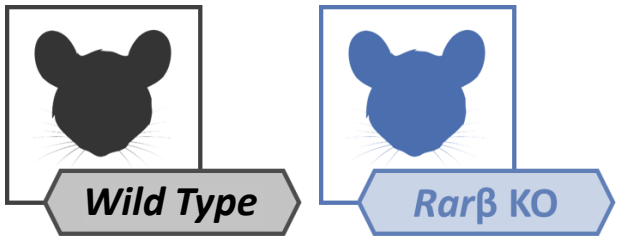


Locomotor Activity



Memory

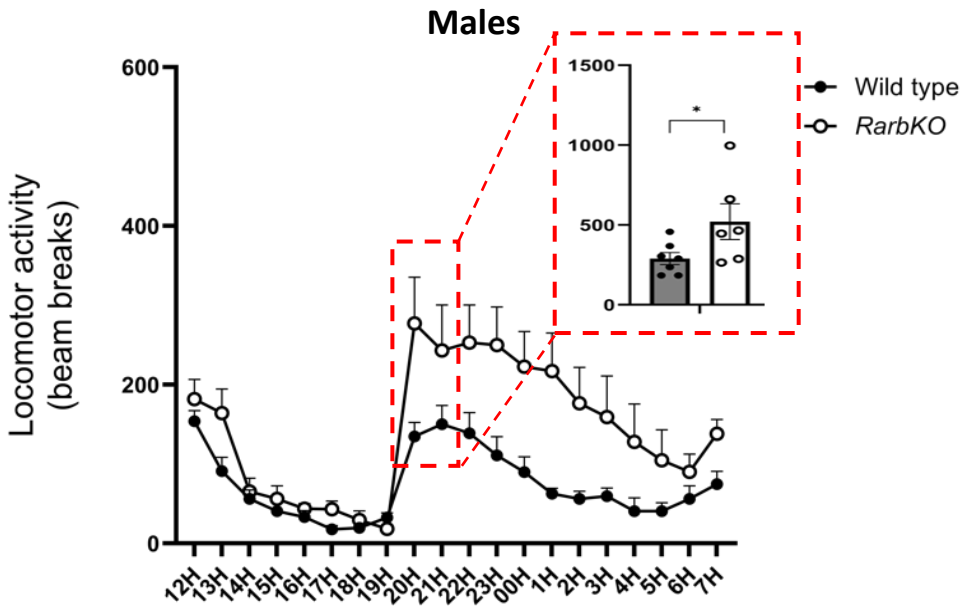
Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior ?

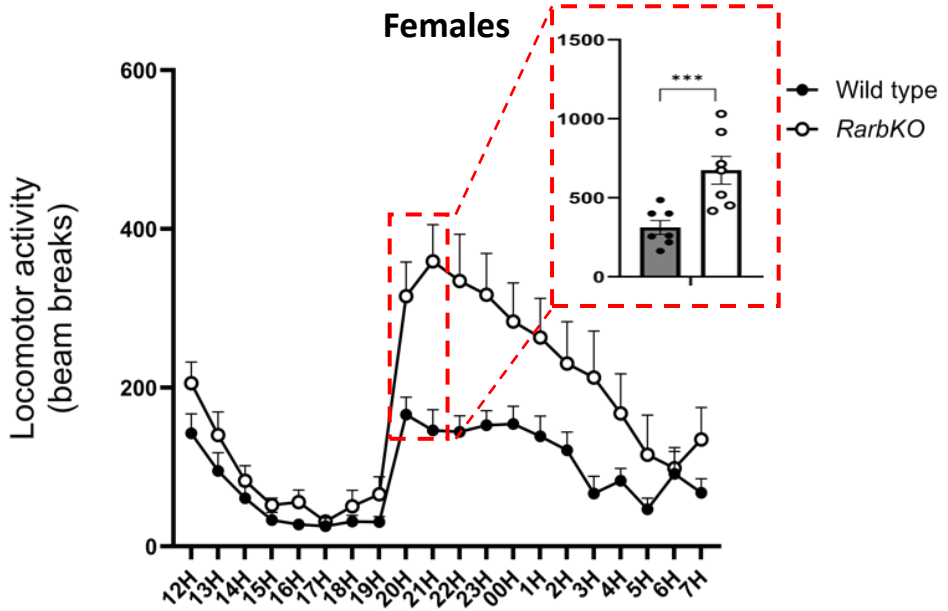


Functional relevance of Glutamatergic signaling upregulation

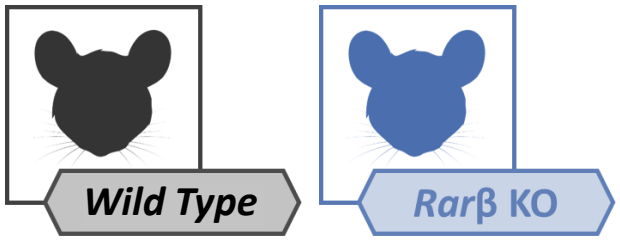


Hyperactivity *of RarβKO* mice during dark phase (Focus on first couple of hours)





Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior ?



NMDA antagonist

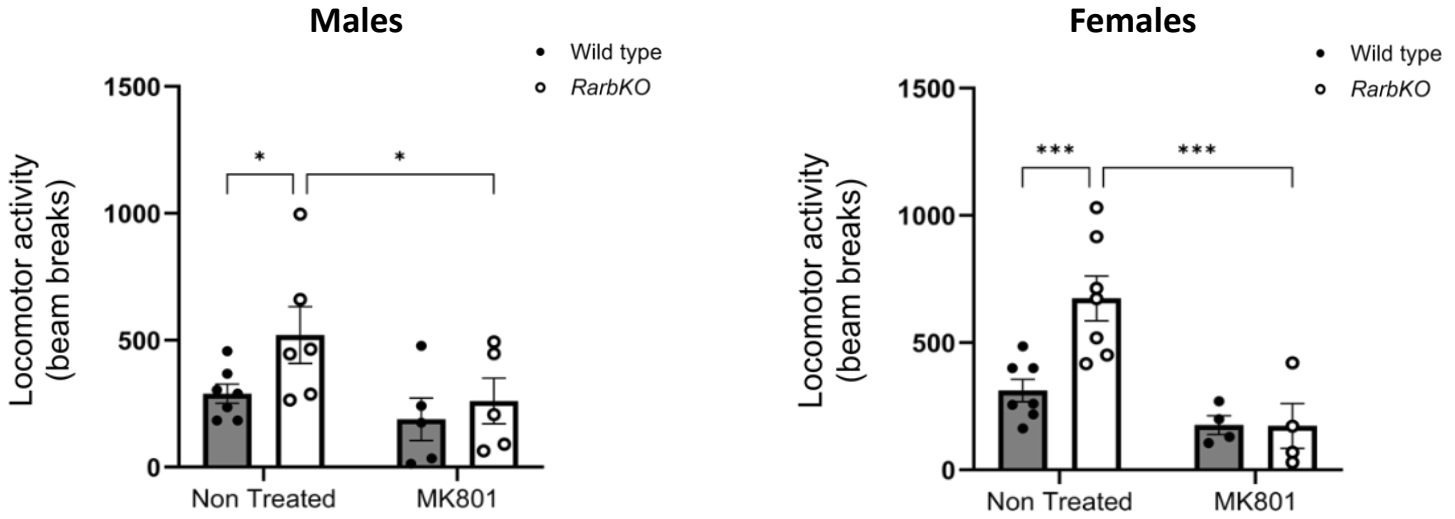


MK801
(Dizocilpine)
Acute treatment
0.2mg/kg

Functional relevance of Glutamatergic signaling upregulation



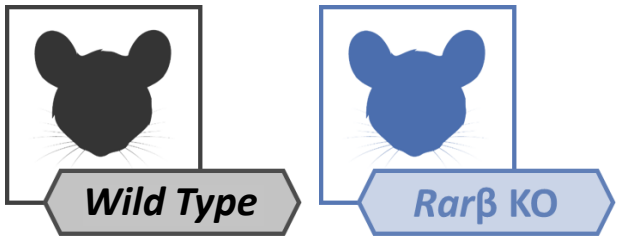
Locomotor Activity



Normalized locomotor activity during first hours of dark phase

Side effects
→Not approved for Human

Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior ?



NMDA antagonist



Memantine
(EBIXA)
Acute treatment
30mg/kg

FDA APPROVED

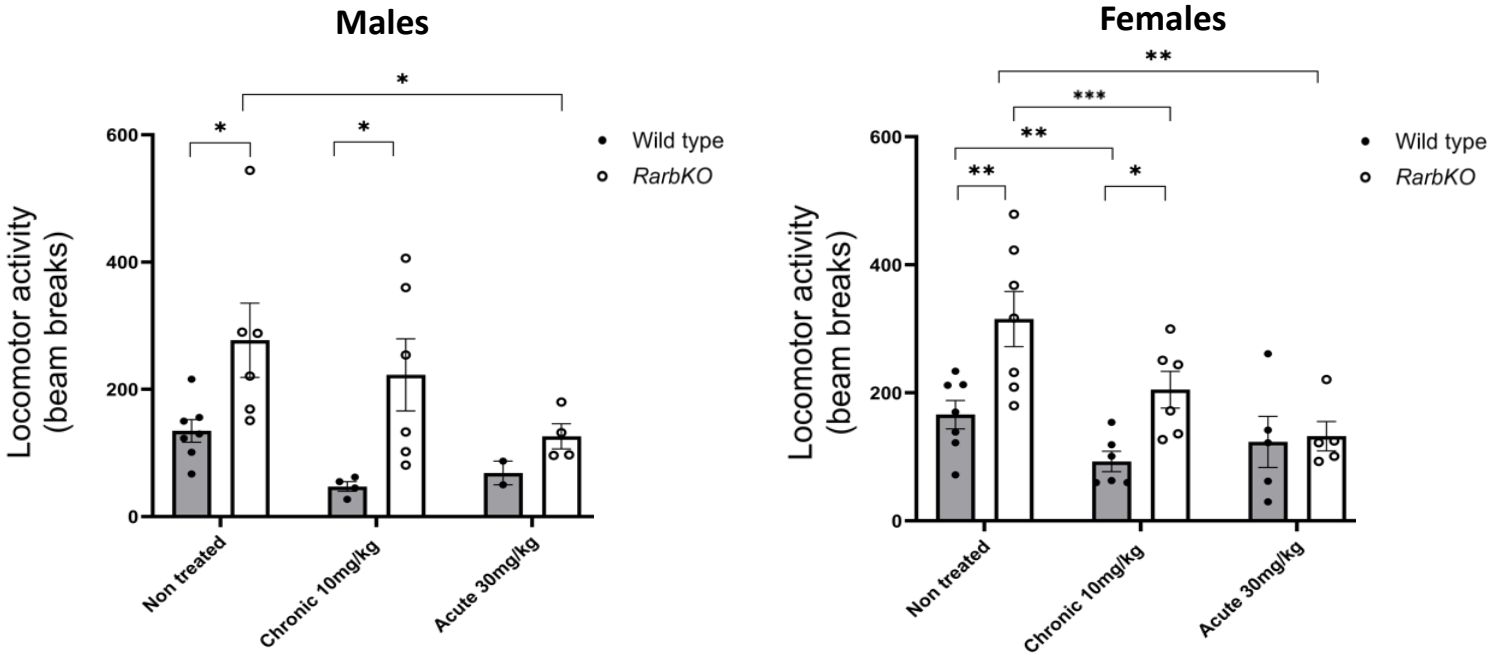


Chronic treatment
10mg/kg

Functional relevance of Glutamatergic signaling upregulation

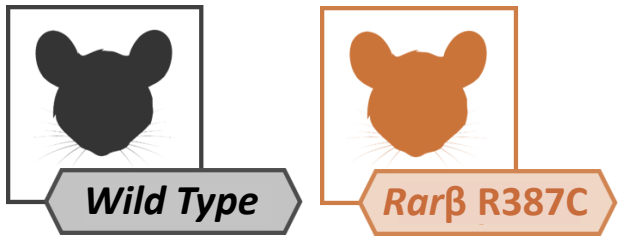


Locomotor Activity



Normalized locomotor activity during first hour of dark phase
Treatment-dependent (Efficacy of Acute high dose treatment)

Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior ?



Functional relevance of Glutamatergic signaling upregulation



Locomotor Activity

NMDA antagonist

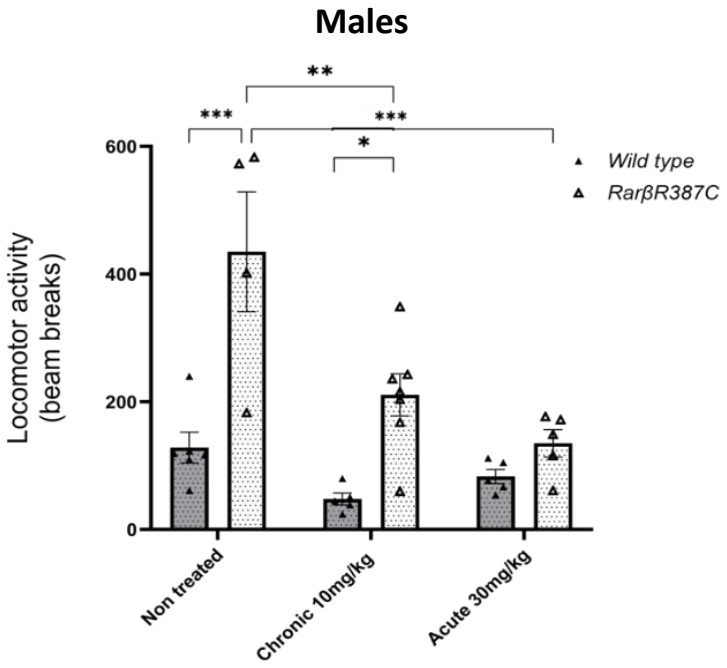


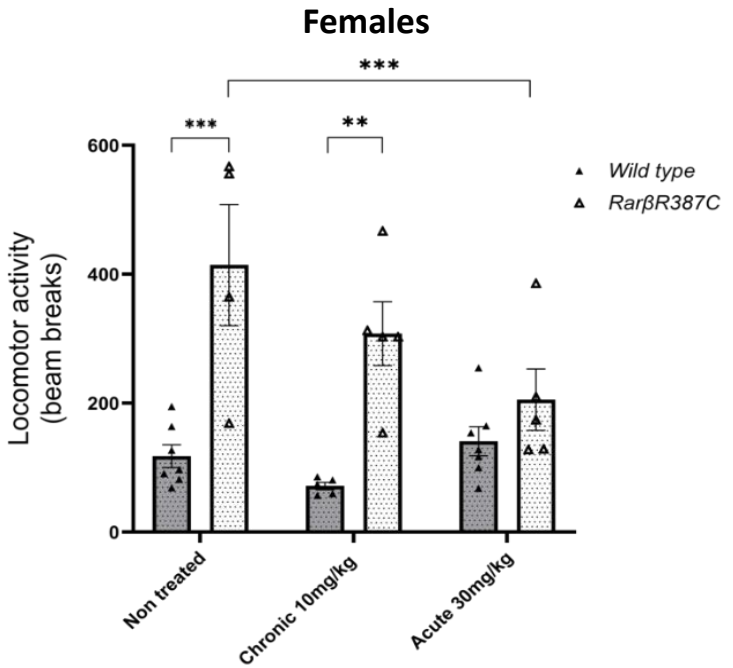
Memantine
(EBIXA)
Acute treatment
30mg/kg

FDA APPROVED



Chronic treatment
10mg/kg

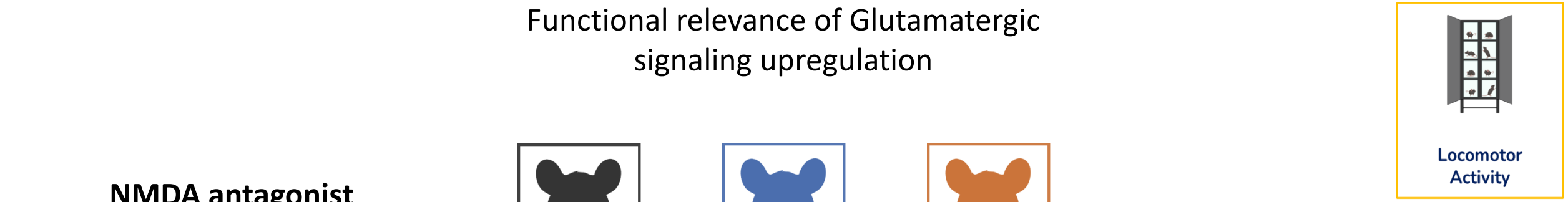




Normalized locomotor activity during first hour of dark phase
Treatment-dependent (Efficacy of Acute high dose treatment)

Treatment - Inhibiting glutamatergic signaling as strategy to normalize behavior

Functional relevance of Glutamatergic signaling upregulation



NMDA antagonist



Memantine
(EBIXA)
Acute treatment
30mg/kg

FDA APPROVED

→ Targeting glutamatergic signaling may offer a potential therapeutic approach for reducing hyperactivity-related symptoms



Chronic treatment
10mg/kg

→ Targeting glutamatergic signaling may offer a potential therapeutic approach for restoring memory deficist



Aseel Madi
(PhD candidate)

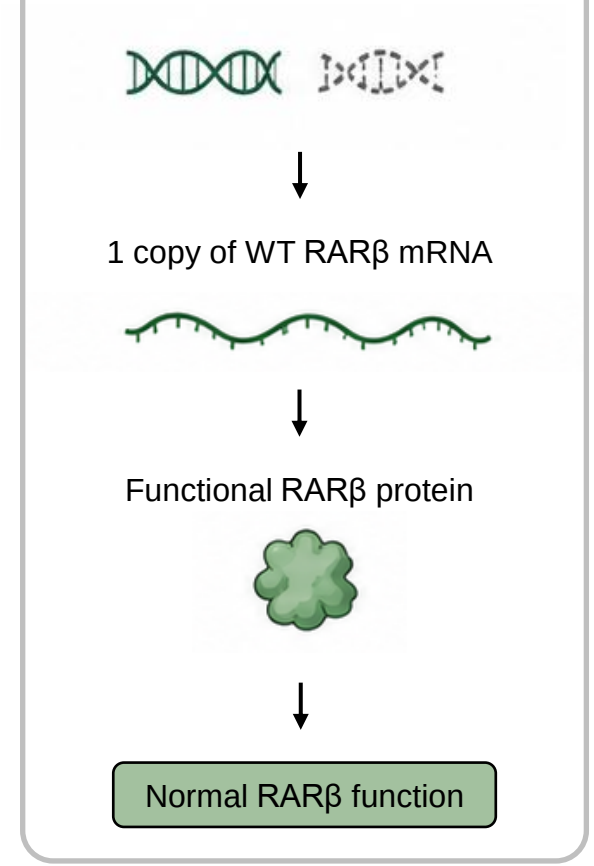
Biomarker study

Cure
MCOPS12

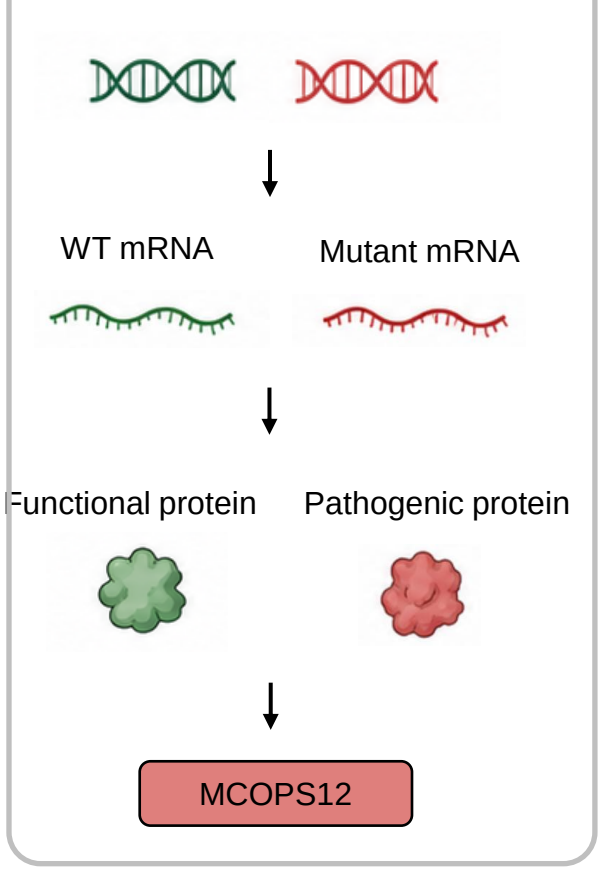
A Cure for Sophia and Friends

Rationale of PhD Project

One functional copy is sufficient

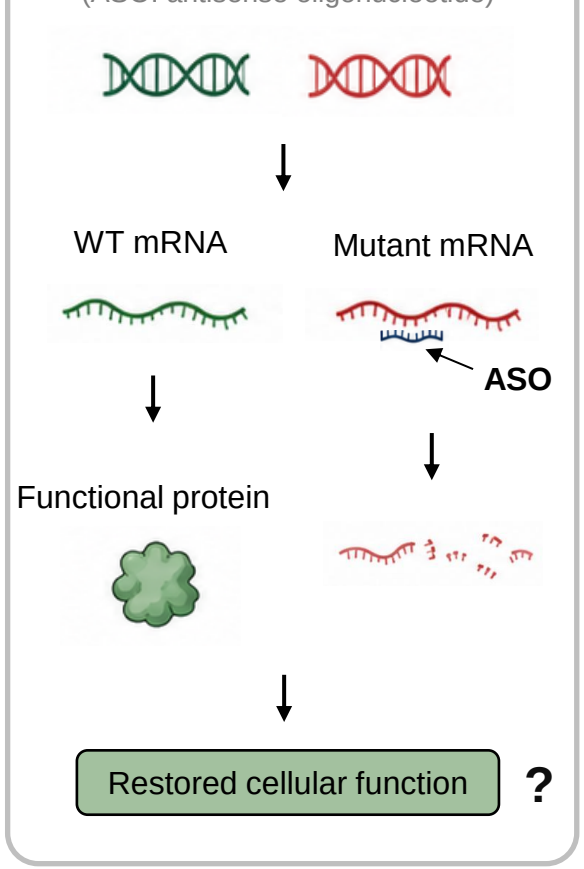


One pathogenic point mutant



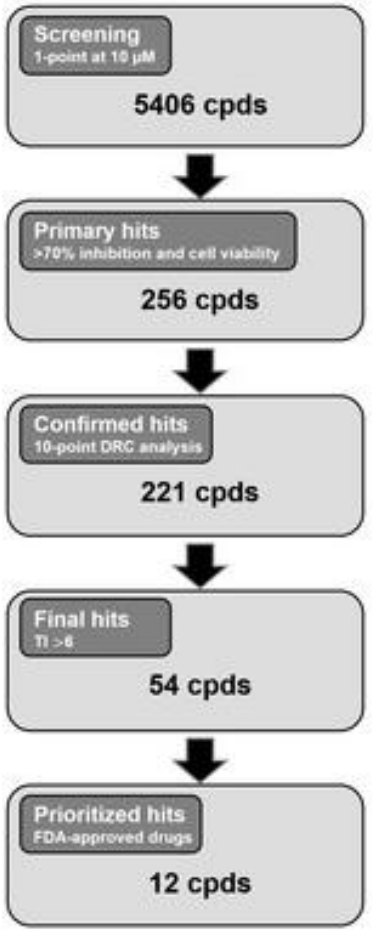
ASO therapeutic strategy

(ASO: antisense oligonucleotide)



Chemolibrary screen

(Simon's neural stem cells)



Limitation: No reliable functional biomarkers for monitoring efficiency of treatment

Objective of PhD Project



Identify biomarkers for ASO efficacy and phenotypic drug screening

- **Lipidomics** — Divi Omics (Mass Spectrometry & Lipidomics, Analytical Services)
- **Metabolomics** — Metabolon
- **Proteomics** — ALAMAR BIOSCIENCES

THANK YOU !!!



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Genomics, IGBMC
Mouse Clinical Institute, IGBMC
Proteomics, IPHC Strasbourg

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